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# Comparative effects of carrageenan on systemic candidiasis and listeriosis in mice

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## SUMMARY

Carrageenan, a toxic substance for the mononuclear phagocyte system (MPS), increases drastically the susceptibility of mice against *Listeria monocytogenes* challenge but induces concurrently an increasing resistance against systemic candidiasis and granulocytosis. These results corroborate the minor role played by MPS, and suggest that polymorphonuclear cells play a major role in non-specific resistance of mice against systemic candidiasis.

## INTRODUCTION

As shown by *in vitro* experiments, the two professional phagocytic cells—polymorphonuclear (PMN) and mononuclear—are able to phagocytose and kill *Candida albicans* (Leijh, van den Barselaar & van Furth, 1977). Many animal models have been developed to determine the respective roles of the PMN and mononuclear phagocyte systems (MPS) *in vivo*. Some resistance has been transferred in dogs against systemic candidiasis by PMN transfusion (Ruthe *et al.*, 1978). In mice, alterations of PMN population induced by immunomodulatory substances have been correlated with candidiasis susceptibility (Hurtrel, Lagrange & Michel, 1980b). On the other hand, in some experimental models, the stimulation of the MPS alone did not confer any protection against systemic candidiasis (Rogers & Balish, 1977; Hurtrel, Lagrange & Michel, 1980b). Nevertheless, the use of classical immunosuppressors or immunostimulants for this purpose is critical and could give conflicting results (Dupont & Drouhet, 1978; Sher *et al.*, 1975; Williams *et al.*, 1978), because, according to the experimental conditions, immunosuppressors for MPS may induce concurrent granulocytopenia and stimulation of the MPS granulocytosis. Recently, it has been shown that carrageenan, a toxic substance for MPS, induces concurrent granulocytosis (Tatsukawa *et al.*, 1979). Thus this treatment which had opposing effects on the two phagocyte cell populations, has been used in mice to determine the respective roles of PMN and MPS in systemic candidiasis.

## MATERIALS AND METHODS

*Mice.* Specific pathogen-free (SPF) Swiss outbred female $OF_1$ mice purchased from IFFA CREDO (Domaine des Oncins, St Germain sur l'Arbresle, France) were used at 6 to 8 weeks of age.

*Candida albicans. C. albicans* were grown twice for 21 hr at 37°C in 10 ml of Sabouraud glucose broth. Yeasts of the second culture were counted in a haemocytometer and then adjusted to a concentration of $2 \times 10^6$ counts per ml with sterile non-pyrogenic saline. Each suspension was

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diluted further and spread onto Sabouraud agar to determine the viable counts which were always $\pm 10\%$ of the haemocytometer counts. For challenge, each mouse received $10^6$ *C. albicans* intravenously (i.v.). Some mice were only used for mortality rate estimation, in other animals, *C. albicans* counts in kidneys were performed 15 hr after challenge. The counts were expressed as $\log_{10}$ and the geometric mean per group was calculated.

*Listeria monocytogenes. L. monocytogenes* obtained from the Institut Pasteur collection (serotype I, no. 54149) were grown twice in tryptase phosphate broth; the second culture obtained after 6 hr was adjusted to a suitable concentration with sterile saline and injected i.v. into mice. Numbers in the inoculum were checked by plating serial dilutions on nutrient agar. Some mice were used only for estimation of mortality rate, in other animals, bacterial counts were performed in spleen and liver 48 hr after challenge. The counts were expressed as $\log_{10}$ and the geometric mean per group was calculated.

*Carrageenan.* Carrageenan (type II, Sigma) was dissolved in sterile non-pyrogenic distilled water and injected intraperitoneally at a dose of 200 mg/kg.

*PMN index.* Peripheral blood counts were performed on blood samples taken from retro-orbital sinus as previously described (Hurtrel, Lagrange & Michel, 1980b). The results are expressed as PMN index, calculated by dividing the absolute number of PMN in animals by the mean found in control mice.

*Mortality rate.* For estimation of mortality rate, animal survivors were checked each day for 3 weeks. During the period after challenge with *C. albicans*, all the animals died; thus mortality is expressed as medial survival time (MST). After challenge with *L. monocytogenes*, death occurred only between days 2 and 7 and mortality is expressed as the percentage of survivors counted 3 weeks after challenge.

*Statistical analysis.* Results are expressed as the arithmetic mean and standard error of the mean. Statistical significance was determined with Student's $t$ -test for non-paired data.

## RESULTS

Mice were pretreated with carrageenan 24 or 48 hr before day of challenge. On this day, control or treated mice were separated into three subgroups; one was challenged with *L. monocytogenes*, another with *C. albicans*, and the last one was used for PMN enumeration. Carrageenan pretreatment drastically increased susceptibility of mice to *L. monocytogenes* (Fig. 1a), almost all carageenan-pretreated animals died and a statistically significant difference in bacteria counts was observed in the spleen ($P < 0{\cdot}01$) and liver ( $P < 0{\cdot}001$) when animals were pretreated 24 hr before, and only at the liver level ($P < 0{\cdot}001$) when mice were pretreated with carrageenan 48 hr before. On the other hand, carrageenan increased resistance of animals against systemic candidiasis (Fig. 1b), it decreased the numbers of *C. albicans* found in kidneys 15 hr after challenge and increased medial survival time, especially in animals pretreated with carrageenan 48 hr before challenge ($P < 0{\cdot}001$). Concurrently, carrageenan induced granulocytosis ($P < 0{\cdot}001$ (Fig. 1c).

## DISCUSSION

Carrageenan exhibits diverse biological properties including activation of Hageman factor (Schwartz & Kellermeyer, 1969), inhibition of complement (Davies, 1965), and macrophage toxicity. Previous *in vitro* work on the cytotoxicity of carrageenan for macrophages (Allison, Harington & Birbech, 1966; Cantanzaro, Schwartz & Graham, 1971) has been recently re-evaluated. Viability and phagocytic functions of newly adherent macrophages seem not to be affected *in vitro* by carrageenan (Simon & Jones, 1978), and *in vivo* histopathological study shows that after i.p. administration, when animals received large doses, carrageenan is not cytotoxic for macrophages except for Kupffer cells in the liver (Sawicki & Catanzaro, 1975). Nevertheless, in guinea-pigs receiving doses of i.p. carrageenan comparative to those used in our experiment, this substance was found 24 hr after inoculation in the macrophages of the spleen red pulp and especially within

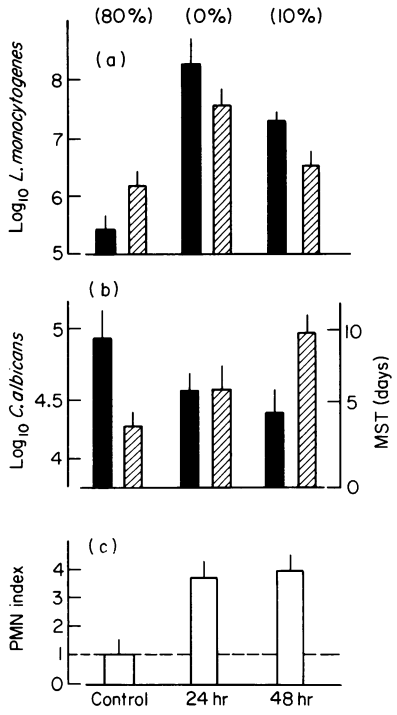


**Fig. 1.** (a) Number of viable *L. monocytogenes* in liver (solid bars) and spleen (shaded bars) 48 hr after challenge, and percentage of survivors (in parentheses), measured in separate groups of mice receiving i.v. $1 \times 10^4$ *L. monocytogenes*, (b) 15-hr *C. albicans* counts in kidneys (solid bars) and medial survival time (MST) (shaded bars), measured in separate groups of mice receiving i.v. $1 \times 10^6$ *C. albicans*, (c) PMN index, in normal (control) and in carrageenan-pretreated mice 24 or 48 hr before. Numbers of *L. monocytogenes*, of *C. albicans* and PMN index were estimated with five mice, MST and percentage of survivors with 10 mice (mean ± standard error of the mean).

the Kupffer cells of the liver (Sawicki & Catanzaro, 1975). It has been shown that polyanionics, such as dextran sulphate (Hahn & Bierther, 1974), carrageenan (Mankes & Abraham, 1975), or sulphatide (Goren *et al.*, 1976), induce alterations in lysosomal function. This may provide a partial explanation for the devastating abrogation by dextran sulphate (Hahn, 1974) and carrageenan (Tatsukawa *et al.*, 1979; Fig. 1a) of defence mechanisms in mice to an i.v. challenge with *L. monocytogenes*.

In spite of its very drastic effect on MPS, carrageenan pretreatment induces some protection in systemic candidiasis which could be seen very early after i.v. challenge at the kidney level, as previously described (Hurtrel, Lagrange & Michel, 1980a), and later on with mortality rate estimation. This protective effect which could be correlated with granulocytosis induced by carrageenan confirms the importance of PMN in systemic candidiasis (Hurtrel, Lagrange & Michel, 1980b). Nevertheless, it must be noted that increasing resistance of mice is highest 48 hr after carrageenan treatment with an equal granulocytosis (Fig. 1c). Depressive action of carrageenan on the PMN blood population but not on the PMN marrow reserve pool released sometimes after carrageenan treatment, or alterations in the plasma factors such as complement which enhanced phagocytosis of the Candida cells (Morelli & Rosenberg, 1971), might explain these differences in resistance found 24 or 48 hr after carrageenan treatment.

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